

**Exhibit A**

# CURRENT PROTOCOLS IN MOLECULAR BIOLOGY

VOLUME 1

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1c. *Harsh treatment:* Pour several hundred milliliters of boiling 0.1% SDS onto the membrane. Cool to room temperature.

*If a membrane is to be reprobed, it must not be allowed to dry out between hybridization and stripping. If it becomes dry, the probe may bind to the matrix.*

2. Place membrane on a sheet of dry Whatman 3MM filter paper and blot excess liquid with a second sheet. Wrap the membrane in plastic wrap and set up an autoradiograph.

*If signal is still seen after autoradiography, rewash using harsher conditions.*

3. The membrane can now be rehybridized. Alternatively, it can be dried and stored for later use.

*Membranes can be stored dry between Whatman 3MM paper for several months at room temperature. For long-term storage, place the membranes in a desiccator at room temperature or 4°C.*

## REAGENTS AND SOLUTIONS

### *Aqueous prehybridization/hybridization (APH) solution*

5× SSC (APPENDIX 2)

5× Denhardt solution (APPENDIX 2)

1% (w/v) SDS

Add 100 µg/ml denatured salmon sperm DNA (see below) just before use

*Alternatives to Denhardt solution and denatured salmon sperm DNA as blocking agents are listed in Table 2.10.5 (see discussion in critical parameters).*

### *Denatured salmon sperm DNA*

Dissolve 10 mg Sigma type III salmon sperm DNA (sodium salt) in 1 ml water. Pass vigorously through a 17-G needle 20 times to shear the DNA. Place in a boiling water bath for 10 min, then chill. Use immediately or store at -20°C in small aliquots. If stored, reheat to 100°C for 5 min and chill on ice immediately before using.

### *Formamide prehybridization/hybridization (FPH) solution*

5× SSC (APPENDIX 2)

5× Denhardt solution (APPENDIX 2)

50% (w/v) formamide

1% (w/v) SDS

Add 100 µg/ml denatured salmon sperm DNA (see above) just before use

*Alternatives to Denhardt solution and denatured salmon sperm DNA as blocking agents are listed in Table 2.10.5 (see discussion in critical parameters).*

*Commercial formamide is usually satisfactory for use. If the liquid has a yellow color, deionize as follows: add 5 g of mixed-bed ion-exchange resin [e.g., Bio-Rad AG 501-X8 or 501-X8(D) resins] per 100 ml formamide, stir at room temperature for 1 hr, and filter through Whatman no. 1 paper.*

**CAUTION:** Formamide is a teratogen. Handle with care.

### *Labeling buffer*

200 mM Tris·Cl, pH 7.5

30 mM MgCl<sub>2</sub>

10 mM spermidine

### *Mild stripping solution*

5 mM Tris·Cl, pH 8.0

2 mM EDTA

0.1× Denhardt solution (APPENDIX 2)

**Exhibit B**

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VOLUME 3

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**SDS electrophoresis buffer, 5x**

15.1 g Tris base  
72.0 g glycine  
5.0 g SDS  
H<sub>2</sub>O to 1000 ml  
Dilute to 1x or 2x for working solution, as appropriate

*Do not adjust the pH of the stock solution, as the solution is pH 8.3 when diluted. Store at 0° to 4°C until use (up to 1 month).*

**SED (standard enzyme diluent)**

20 mM Tris·Cl, pH 7.5  
500 µg/ml bovine serum albumin (Pentax Fraction V)  
10 mM 2-mercaptoethanol  
Store up to 1 month at 4°C

**Sodium acetate, 3 M**

Dissolve 408 g sodium acetate·3H<sub>2</sub>O in 800 ml H<sub>2</sub>O  
Add H<sub>2</sub>O to 1 liter  
Adjust pH to 4.8 or 5.2 (as desired) with 3 M acetic acid

**Sodium acetate buffer, 0.1 M**

*Solution A:* 11.55 ml glacial acetic acid/liter (0.2 M).  
*Solution B:* 27.2 g sodium acetate (NaC<sub>2</sub>H<sub>3</sub>O<sub>2</sub>·3H<sub>2</sub>O)/liter (0.2 M).

Referring to Table A.2.2 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H<sub>2</sub>O to 100 ml. (See Potassium acetate buffer recipe for further details.)

**Sodium phosphate buffer, 0.1 M**

*Solution A:* 27.6 g NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O per liter (0.2 M).  
*Solution B:* 53.65 g Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O per liter (0.2 M).

Referring to Table A.2.3 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H<sub>2</sub>O to 200 ml. (See Potassium phosphate buffer recipe for further details.)

**SSC (sodium chloride/sodium citrate), 20x**

3 M NaCl (175 g/liter)  
0.3 M Na<sub>3</sub>citrate·2H<sub>2</sub>O (88 g/liter)  
Adjust pH to 7.0 with 1 M HCl

**STE buffer**

10 mM Tris·Cl, pH 7.5  
10 mM NaCl  
1 mM EDTA, pH 8.0

**TAE (Tris/acetate/EDTA) electrophoresis buffer**

<i>50x stock solution:</i>	<i>Working solution, pH ~8.5:</i>
242 g Tris base	
57.1 ml glacial acetic acid	40 mM Tris·acetate
37.2 g Na <sub>2</sub> EDTA·2H <sub>2</sub> O	2 mM Na <sub>2</sub> EDTA·2H <sub>2</sub> O
H <sub>2</sub> O to 1 liter	

**TBE (Tris/borate/EDTA) electrophoresis buffer**

*10x stock solution, 1 liter:*  
108 g Tris base (890 mM)  
55 g boric acid (890 mM)  
40 ml 0.5 M EDTA, pH 8.0 (20 mM)

**Appendix 2**

**A.2.5**